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From: Nguyen, Quang (AU1632)
Sent: Friday, June 07, 2002 11:39 AM
To: STIC-ILL
Subject: Reference request for 09/846588

I would like to request the following references which are not available at US PTO URGENTLY.

(1) Pencea V et al. Society of Neuroscience Abstracts 25:2045, 1999. (812.11)

(2) Benraiss A et al. Society of Neuroscience Abstracts 25:1028, 1999.

(3) Goldman SA. Journal of Neurobiology 36:267-286, 1998.

THANK YOU.

812.9

INHIBITOR OF ENDOTHELIN CONVERTING ENZYME AFFECTS BRAIN DEVELOPMENT IN CHICK EMBRYOS. G. Rizzi,[1] A. Y. Jeng,[2] and L. Hsu[1]*. [1]Department of Biology, Seton Hall University, S. Orange, NJ 07079; [2]Novartis Institute for Biomedical Research, Summit, NJ 07901.

Endothelins are potent vasoconstrictive peptides that have mitogenic effects on glial or smooth muscle cells. Exogenous administration of endothelin-1 (ET-1) on day 5 in developing embryonic chicks consistently increased body and brain dimensions. Binding studies also indicated that [^{125}I]ET-1 bound maximally to membranes from brains of 5 day old embryos, suggesting that the growth promoting effects of ET-1 are age-dependent. To establish the physiological role of endogenous ET-1 in development, an inhibitor of endothelin converting enzyme that catalyzes the final step of post-translational processing of ET-1 was administered to embryonic chicks on day 4. The effects of the inhibitor, CGS 26303, was dose-dependent. At 40-60 uM, it significantly reduced both body and brain dimensions. To study the effects on neurogenesis, 4 day old embryos were treated with CGS 26303 followed by incubation with bromodeoxy-uridine (Brdu) to label cells undergoing DNA synthesis. Immunocyto-chemical localization of Brdu by monoclonal antibody against Brdu in serial paraffin sections of optic lobes of inhibitor-treated embryos showed a decrease in labelled cells in the neuroepithelial germinal layer when compared to controls. These results are consistent with our hypothesis that ET-1 plays a growth promoting role during brain development.

812.10

PERIPHERAL INFUSION OF IGF-I SELECTIVELY INDUCES NEUROGENESIS IN THE ADULT RAT HIPPOCAMPUS. Maria A.I. Åberg[1], N. David Åberg[1], Helena Hedbäcker[1], Jan Oscarsson[2], Henrik Hagberg[3]* and Peter S. Eriksson[1]. Institute of Clinical Neuroscience[1], Department of Physiology[2], Dept of Obstetrics and Gynaecology[3], University of Göteborg, 41345 Göteborg, Sweden

The ability to pharmacologically regulate adult neurogenesis is of potentially important therapeutic value. The aim of the present project was to investigate the effect of insulin-like growth factor-I (IGF-I) on proliferation and differentiation of adult rat hippocampal neural progenitor cells. Using bromodeoxyuridine (BrdU) labeling, we found an increase in the number of BrdU-immunoreactive progenitors after IGF-I treatment. Furthermore, by using double-immunoflourescence staining for BrdU and cell-specific markers, we found that peripheral infusion of IGF-I selectively induces neurogenesis in progenitor progeny, with a resulting 78% net increase in the number of newly generated neurons in the granule cell layer (GCL) in the adult dentate gyrus over that of controls.

In conclusion, the evidence for a selective induction of neurogenesis by IGF-I on hippocampal progenitors in vivo suggests an important role for IGF-I in the generation of new neurons in the adult hippocampus. This and additional studies concerning the effects of IGF-I in other adult brain areas would hopefully offer therapeutic strategies for several neurodegenerative diseases. This study was supported by grants from the Swedish Medical Research Council (project no K98-12X-12535-01A).

812.11

INFUSION OF BDNF INTO THE LATERAL VENTRICLE OF THE ADULT RAT LEADS TO AN INCREASE IN THE NUMBER OF NEWLY GENERATED CELLS IN THE FORE, MID AND HINDBRAIN PARENCHYMA. V. Pencea[1]*, K.D. Bingaman[1,2], S.J. Wiegand[3] and M.B. Luskin[1]. [1]Depts. of Cell Biology & [2]Neurosurgery, Emory Univ. Sch. of Med., Atlanta, GA 30322 and [3]Regeneron Pharmaceuticals, Inc., Tarrytown, NY 10591.

Our previous studies have shown that infusion of BDNF into the lateral ventricle of adult rats resulted in an increased number of newly-generated neuronal progenitor cells in the rostral part of the subventricular zone (SVZa) and new neurons in the olfactory bulb (OB). The aim of this study was to determine whether a similar BDNF infusion influenced the production of cells around the lateral, 3rd and 4th ventricles. Adult rats received a continuous intraventricular infusion of BDNF (12 µg/d) and the cell proliferation marker BrdU (12 µg/d) in PBS for a 12 day interval and were perfused 16 days after the BDNF/BrdU withdrawal. In the control animals the BDNF was omitted. In the brains of the BDNF-infused animals there was a substantial number of BrdU(+) cells not only in the SVZa and OB, but also in other forebrain structures in the vicinity of the infused ventricle, including the striatum, septum, corpus callosum and cerebral cortex. There was also prominent BrdU labeling in the adjoining contralateral septum and corpus callosum. In addition, the BDNF infusion led to an increase in the number of BrdU(+) cells bilaterally in the parenchyma surrounding the 3rd and 4th ventricles. A subset of the BrdU(+) cells in all regions analyzed expressed a neuronal phenotype. In the PBS-infused brains the distribution of BrdU(+) cells was similar, but their number was lower. Our results suggest that many regions of the CNS harbor progenitor cells that give rise to neurons. (Supported by NIDCD and Regeneron)

812.12

Separate proliferation kinetics of FGF-responsive and EGF-responsive neural stem cells within the embryonic forebrain germinal zone. David J. Martens*, Vincent Tropepe & Derek van der Kooy. University of Toronto, Department of Anatomy and Cell Biology, Toronto, Ontario

The embryonic forebrain germinal zone contains two separate and additive populations of stem cells that both exhibit self-renewal and multipotentiality. While cumulative S-phase labeling studies have investigated the proliferation kinetics of the overall population of precursor cells within the forebrain germinal zone through brain development, little is known about when and how (symmetrically or asymmetrically) the small populations of stem cells are proliferating *in vivo*. This has been determined by injecting timed-pregnant mice with high doses of tritiated thymidine (^{3}H-thy) to kill any stem cells proliferating within the striatal germinal zone *in vivo* and then by assaying for neurosphere formation *in vitro*. Injections of 0.8 mCi of ^{3}H-thy given every 2 h for 12 h to timed-pregnant mice at E11, E14, and E17 resulted in significant depletions in the number of neurospheres generated by FGF-responsive stem cells at E11, and by EGF-responsive and FGF-responsive stem cells at E14 and E17. With increasing embryonic age, the depletions observed in the number of neurospheres generated *in vitro* in response to FGF2 following exposure to ^{3}H-thy *in vivo* decreased, suggesting there is an increase in the length of the cell cycle of FGF-responsive neural stem cells through embryonic development. The results suggest that the FGF-responsive stem cell population expands between E11 and E14 by dividing symmetrically, but switches to primarily asymmetric division between E14 and E17. The EGF-responsive stem cells arise after E11, and their population expands through symmetric divisions and through asymmetric divisions of FGF-responsive stem cells. (Supported by MRC of Canada and the MS Society of Canada).

812.13

SERUM-FREE N2 MEDIA AND BASIC FIBROBLAST GROWTH FACTOR INDUCE PROLIFERATION IN BOVINE NEONATAL CHROMAFFIN CELLS. E.D. Potter*, W.M. Walters, B.R. Frydel, X.T. Nie, J. Huang, and J. Sagen. The Miami Project to Cure Paralysis, Univ. Miami Sch. Med., Miami, FL 33136

While pluripotent stem cells have been described in fetal chromaffin cell cultures, this population is significantly decreased in cultures isolated from neonatals and adults. Fetal chromaffin cells are difficult to procure, creating an obstacle for studies describing their differentiation and potential use in transplantation therapies. Thus we investigated cultivation of neonatal bovine chromaffin cells in N2 media supplemented with basic fibroblast growth factor (bFGF). This serum-free N2/bFGF media promotes viability and proliferation of stem cells isolated from the CNS. Bovine chromaffin cells were isolated from 1-5 day old calf adrenals and cultivated in serum free N2 media supplemented with bFGF(10ng/ml). After 5 days cultivation, numerous nestin-positive and bromo-deoxyuridine (BrdU)-positive proliferating spheres appeared. After 3 weeks cultivation in N2/bFGF alone, or in N2/bFGF supplemented with 5 µM dexamethasone, 10% fetal bovine serum (FBS), or both, all cultures contained numerous BrdU-positive cells that were also immunoreactive for tyrosine hyroxylase, dopamine-β-hydroxylase, phenylethanolamine-N-methyl transferase, and chromagranin. Numerous cells cultivated in N2/bFGF in the presence of 10% FBS developed processes and a neuronal morphology. HPLC analysis demonstrated release of norepinepherine and epinepherine from sister cultures. Preliminary studies indicate that N2/bFGF can also induce proliferation of neonatal porcine chromaffin cells. These results suggest the possibility of maintaining continuous chromaffin cells for transplantation. Supported by NS25054.

812.14

THE EFFECTS OF LIF FAMILY CYTOKINES ON HUMAN NEURAL PROGENITOR CELL CULTURES. X. Cui[1], Å. Seiger[2], P. Almqvist[1], and L. Wahlberg[1]*. [1]Dept. of Clinical Neuroscience, Section of Neurosurgery, Karolinska Institute, 171 76 Stockholm, Sweden; [2]Dept. of Geriatric Medicine, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden.

We have previously shown that human neural progenitor cell cultures derived from the first trimester central nervous system (CNS) can be expanded > 1 yr. using defined serum free medium supplemented with epidermal growth factor (EGF), fibroblast growth factor (bFGF), and leukemia inhibitory factor (LIF). LIF belongs to the neuropoietic cytokine family, which includes ciliary neurotrophic factor (CNTF) and interleukine-6 (IL-6). In this study, the comparative effects of these cytokines on embryonic human CNS progenitor cultures were examined. We found that cultures can be grown for > 6 mos. under the following four conditions: EGF/bFGF, EGF/bFGF/IL-6, EGF/bFGF/LIF, and EGF/bFGF/CNTF. Even though growth was achieved in all conditions, the growth rates for the cells cultured with EGF/bFGF/LIF and EGF/bFGF/CNTF were much greater than that of cells cultured with EGF/bFGF and EGF/bFGF/IL-6. Upon plating and differentiation, the progenitors from all conditions differentiated into the three major neural phenotypes, neurons, astrocytes and oligodendrocytes. Progenitors expanded with EGF/bFGF/LIF or EGF/bFGF/CNTF gave rise to more neurons than with EGF/bFGF or EGF/bFGF/IL-6. The proportion of phenotypes remained stable over a relatively long period of time (> 90 DIV) under all culture conditions. Consistent with the proliferation data, western blot analysis showed that both LIFR and CNTFRα are expressed but no IL-6R was detected. In conclusion, embryonic human neural progenitors can be expanded using EGF/bFGF and even though LIF or CNTF signaling are not required for proliferation, the expansion rate is greatly enhanced when these factors are added. Interestingly, both LIF and CNTF increased the proportion of neurons seen during *in vitro* differentiation. *This study was supported by CytoTherapeutics, Inc.*